

Ca 23
Cs 1

S AFNTQAAP 31
G 1

Tr2#		10	20	30	40	
Bp	1	RTITNNEMGN	HSGYDYELWK	DYGNT-SMTL	NNGGAFSAGW	N--NIGNA 45
Ca	32	KTITSNEIGV	NGGYDYELWK	DYGNT-SMTL	KNGGAFSCQW	S--NIGNA 76
Fs	1	NSSVTGNVG	SSPYHYEIWY	QGG-NNSMTF	YDNGTYKASW	N--GTNDF 44
Cs	2	RIIYDNETGT	HGGYDYELWK	DYGNT-IMEL	NDGGTFSCQW	S--NIGNA 46
Rf	1	SAADQQTRGN	VGGYDYEMWN	QNGGQASMN	PGAGSFTCSW	S--NIENF 46
Tr2	1	QTIQPGTGY	NNGYFYSYWN	DGHGGVITYN	GPGGQFSVNW	S--NSGNF 45
Tv	1	QTIQPGTGF	NNGYFYSYWN	DGHGGVITYN	GPGGQFSVNW	S--NSGNF 45
Th	1	QTIGPGTGY	SNGYFYSYWN	DGHAGVITYN	GCGGSFTVNW	S--NSGNF 45
Sc	1	SGTPSSSTGT	DGGYFYSWWT	DGAGDATYQN	NGGGSYTLTW	SG--NNGNL 46
An	1	S	AGINYVQNYN	GNLGDFTY-D	ESAGTFSMYW	EDGVSSDF 38
Ak	1	S	AGINYVQNYN	GNLADFTY-D	ESAGTFSMYW	EDGVSSDF 38
AT	1	S	AGINYVQNYN	QNLGDFTY-D	ESAGTFSMYW	EDGVSSDF 38
Tr1	1		ASINYDQNYQ	TGG-QVSY-	PSNTGFSVNW	N--TQDDF 34
Aa	1	RSTPSSTGE	NNGYFYSFWT	DGGGDVITYN	GNAGSYSVEW	S--NVGNF 45
Ss	1	ATTIT-NETGY	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTRW	T--NCGNF 45
SlB	1	DTVVTNQEQT	NNGYFYSFWT	DSQGTVMNM	GSGGQYSTSW	R--NTGNF 47
SlC	1	ATTITTNETGT	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTQW	T--NCGNF 46
Tl	1	QTPNSEGW	HDGYFYSWWS	DGGAQATYTN	LEGGTYEISW	G--DGGNL 45
Tf	1	AVTSNETGY	HDGYFYSFWT	DAPGTVSMEL	GPGGNYSYSW	R--NTGNF 45
Bc	1		ASTDYWQNW	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF 36
Bs	1		ASTDYWQNW	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF 36

FIGURE 1

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Tr2#		50		60		70		80	
Bp	46	LFRK-GKKFD	ST-RTHHQLG	NISINYNASF	N-PSGNSYLC	VYGWTQSP		90	
Ca	77	LFRK-GKKFN	DT-QTYKQLG	NISVNYNCNY	Q-PYGN SYLC	VYGWTSSP		121	
FS	45	LARV-GFKYD	EK-HTYEELGP	IDAYYKWSKQ	GSAGGYNYIG	IYGWTVDP		91	
Cs	47	LFRK-GRKFN	SD-KTYQELG	DIVVEYGCDY	N-PNGNSYLC	VYGWTRNF		91	
Rf	47	LARM-GKNYD	SQKKNYKAFG	NIVLTYDVEY	T-PRGNSYMC	VYGWTRNP		92	
Tr2	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNNGNSYLS	VYGWSRNP		83	
Tv	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNNGNSYLS	VYGWSRNP		83	
Th	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNNGNSYLS	IYGWSRNP		83	
Sc	47	VGGK-GWNPG	AASRS-----	---ISYS-GT	YQPNNGNSYLS	VYGWTRSS		84	
An	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYP		77	
Ak	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYP		77	
At	39	VVGLGWTG	SSNA-----	---ITYSAEY	SASGSASYLA	VYGVVNYP		77	
Tr1	35	VVGW-GWTTG	SSAP-----	---INFGGSF	SVNSGTGLLS	VYGWSTNP		72	
Aa	46	VGGK-GWNPG	SAKD-----	---ITYSGNF	T-PSGNGYLS	VYGWTDDP		83	
Ss	46	VAGK-GWANG	GR-RT-----	---VRYT-GW	FNPSGNGYGC	LYGWTSNP		82	
SlB	48	VAGK-GWANG	GR-RT-----	---VOYS-GS	FNPSGNAYLA	LYGWTSNP		84	
SlC	47	VAGK-GWSTG	DGN-----	---VRYN-GY	FNPVGNGYGC	LYGWTSNP		82	
Tl	46	VGGK-GWNPG	LNARA-----	---IHFE-GV	YQPNNGNSYLA	VYGWTRNP		83	
Tf	46	VAGK-GWATG	GR-RT-----	---VTYS-AS	FNPSGNAYLT	LYGWTRNP		82	
Bc	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGWTRSP		75	
Bs	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGWTRSP		75	

Tr2#		90		100		110		120		130
Bp	91	LAEYYIVDSW	GTyr-PT--G	AYKGSFYADG	GTyDIYETTR	VNOPSIIIG		135		
Ca	122	LVEYYIVDSW	GSRPP--GG	TSKGTITVDG	GIYDIYETTR	INOPSIOG		167		
FS	92	LVEYYIVDDW	FNKPGANLLG	QRKGEFTVDG	DTYEIWQNT	VQOPSIKG		139		
Cs	92	LVEYYIVESW	GSRPP--GA	TPKGTITQWMAG	TYEIYETTR	VNOPSIDG		138		
Rf	93	LMEYYIVEGW	GDWRPPGNDG	EVKGTVSANG	NTYDIRKTMR	YNOPSLDG		140		
Tr2	84	LIEYYIVENF	GTYN-PSTGA	TKLGEVTS DG	SVYDIYRTOR	VNOPSIIIG		130		
Tv	84	LIEYYIVENF	GTYN-PSTGA	TKLGEVTS DG	SVYDIYRTOR	VNOPSIIIG		130		
Th	84	LIEYYIVENF	GTYN-PSTGA	TKLGEVTS DG	SVYDIYRTOR	VNOPSIIIG		130		
Sc	85	LIEYYIVESY	GSYD-PSSAA	SHKGSVTCNG	ATYDILSTWR	YNAPSIDG		131		
An	78	GAEYYIVEDY	GDYN-PCSSA	TSLGTVYS DG	STYQVCTDTR	INEPSITG		124		
Ak	78	QAEYYIVEDY	GDYN-PCSSA	TSLGTVYS DG	STYQVCTDTR	TNEPSITG		124		
At	78	QAEYYIVEDY	GDYN-PCSSA	TSLGTVYS DG	STYQVCTDTR	INEPSITG		124		
Tr1	73	LVEYYIMEDN	HNY--PAQ-G	TVKGTVTS DG	ATYTIWENTR	VNEPSIOG		117		
Aa	84	LIEYYIVESY	GDYN-PGSGG	TTRGNVSS DG	SVYDIYTATR	TNAPSIDG		130		
Ss	83	LVEYYIVDNW	GSYR-PT--G	ETRGTVHSDG	GTyDIYKTTR	YNAPSVEA		127		
SlB	85	LVEYYIVDNW	GTyr-PT--G	EYKGTVTS DG	GTyDIYKTTR	VNKPSVEG		129		
SlC	83	LVEYYIVDNW	GSYR-PT--G	TYKGTVSS DG	GTyDIYQTTR	YNAPSVEG		127		
Tl	84	LVEYYIVENF	GTyD-PSSGA	TDLGTVECDG	SIYRLGKTTR	VNAPSIDG		130		
Tf	83	LVEYYIVESW	GTyr-PT--G	TYMGTVTT DG	GTyDIYKTTR	YNAPSIEG		127		
Bc	76	LIEYYVVD SW	GTyr-PT--G	TYKGTVKSDG	GTyDIYTTR	YNAPSIDG		120		
Bs	76	LIEYYVVD SW	GTyr-PT--G	TYKGTVKSDG	GTyDIYTTR	YNAPSIDG		120		

FIGURE 1 CONT'D

Tr2#		140	150	160	
Bp	136	-IATFKQYWS VRQTKRTS--	-----GTVS	VSAHFRKWES	LGMPL-GK 174
Ca	168	-NTTFKQYWS VRRTKRTS--	-----GTIS	VSKHFAAWES	KGMPL-GK 206
Fs	140	-TQTFQYFWS VRKSARSC--	-----GHID	ITAHMKKWE	LGMKM-GK 178
Cs	139	-TATFQYFWS VRTSKRTS--	-----GTIS	VTEHFKQWER	MGMRM-GK 177
Rf	141	-TATFQYFWS VRQTSQSANN	QTNMKGTID	VSKHFDAS	AGLDMSGT 187
Tr2	131	-TATFYQYWS VRRNHR-S-S	-----GSVN	TANHFNAAQ	QGLTL-GT 168
Tv	131	-TATFYQYWS VRRTHR-S-S	-----GSVN	TANHFNAAQ	QGLTL-GT 168
Th	131	-TATFYQYWS VRRNHR-S-S	-----GSVN	TANHFNAAQ	HGLTL-GT 168
Sc	132	-TQTFQYFWS VRNPKKAPGG	SIS---GTV	VOCHFDK	LGMNLGSE 175
An	125	-TSTFTQYFS VRESTRTS--	-----GTVT	VANHFNFAQ	HGFGN-SD 163
Ak	125	-TSTFTQYFS VRESTRTS--	-----GTVT	VANHFNFAQ	HGFGN-SD 163
At	125	-TSTFTQYFS VRESTRTS--	-----GTVT	VANHFNFAH	HGFHN-SD 163
Tr1	118	-TATFNQYIS VRNSPR-T-S	-----GTVT	VQNHFNWAS	LGLHLGQM 155
Aa	131	-TQTFQYFWS VRONKR-VG-	-----GTVT	TSNHFNAAK	LGMNL-GT 168
Ss	128	-PAAPDQYWS VRQSKVT--S	-----GTIT	TGNHFDAAW	AGMNMGNF 168
SlB	130	TR-TFDQYWS VRQSKR-TG-	-----GTIT	TGNHFDAAW	AGMPLGNF 168
SlC	128	TK-TFQYFWS VRQSKVTSGS	-----GTIT	TGNHFDAAW	AGMNMGQF 168
Tl	131	TQ-TFDQYWS VRQDKR-T-S	-----GTVQ	TGCHFDAAW	AGLNVNGD 169
Tf	128	TR-TFDQYWS VRQSKRTS--	-----GTIT	AGNHFDAAW	HGMHLGTH 166
Bc	121	DRTTFTQYWS VRQSKRPTGS	N-----ATIT	FTNHVNAWS	HGMNLGSN 163
Bs	121	DRTTFTQYWS VRQSKRPTGS	N-----ATIT	FSNHVNAWS	HGMNLGSN 163

Tr2#		170	180	190	
Bp	175	MYETAFTVEG	YQSSGSANVM	TNQLFIGN	201
Ca	207	MHETAFNIEG	YQSSGKADV	SMSINIGK	233
Fs	179	MYEAKVLVEA	GGSQSFDV-	TYFKMT	203
Cs	178	MYEVALTVEG	YQSSGYANVY	KNEIRIGANP....	
Rf	188	LYEVSLNIEG	YRSNGSANVK	SVSV	211
Tr2	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Tv	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Th	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Sc	176	HNYQIVATEG	YQSSGTATI-	TVT	197
An	164	FNYQVMAVEA	WSGAGSASV-	TISS	185
Ak	164	FNYQVMAVEA	WSGAGSASV-	TISS	185
At	164	FNYQVMAVEA	WSGAGSAAV-	TISS	185
Tr1	157	MNYQVMAVEG	WGSAGSASQ-	SVSN	178
Aa	169	HNYQILATEG	YQSSGSSSI-	TIQ	190
Ss	167	RYYMINATEG	YQSSGSSTI-	TVSG	189
SlB	169	SYMINATEG	YQSSGTSSI-	NVGG.....	
SlC	169	RYYMINATEG	YQSSGSSNI-	TVSG	191
Tl	170	HYYQIVATEG	YFSSGYARI-	TVADV	194
Tf	167	D-YMIMATEG	YQSSGSSNVT	LGTS.....	
Bc	164	WAYQVMATEG	YQSSGSSNV-	TVW	185
Bs	164	WAYQVMATEG	YQSSGSSNV-	TVW	185

FIGURE 1 CONT'D

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Bp *Bacillus pumilus*
Ca *Clostridium acetobutylicum* P262 XynB
Cs *Clostridium stercorarium* xynA
Rf *Ruminococcus flavefaciens*
Tr2 *Trichoderma reesei* XYN II
Tv *Trichoderma viride*
Th *Trichoderma harzianum*
Sc *Schizophyllum commune* Xylanase A
An *Aspergillus niger*, var. *awamori*
Ak *Aspergillus kawachii* XynC
At *Aspergillus tubigensis*
Tr1 *Trichoderma reesei* XYN I
Aa *Aspergillus awamori* var. *kawachi* Xyn B
Fs *Fibrobacter succinogenes* XYN II
Ss *Streptomyces* sp. 36a
SlB *Streptomyces lividans* Xln B
SlC *Streptomyces lividans* Xln C
Tl *Thermomyces lanuginosus* Xyn
Tf *Thermomonospora fusca* TfxA
Bc *Bacillus circulans*
Bs *Bacillus subtilis*

FIGURE 1 CONT'D

[illegible]

5' -CT AGC TAA GGA GG CTG CAG ATGst
 G ATT CCT CC GAC GTC TAC
 NheI | PstI

 TrX-1
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
 Q T I Q P G T G Y N N G Y F Y S
 CAA ACA ATA CAA CCA GGA ACC GGT TAC AAC AAC GGT TAC TTT TAC AGC
 GTT TGT TAT GTT GGT CCT TGG CCA ATG TTG TTG CCA ATG AAA ATG TCG
 TrX-8 AgeI

 XyTv-2
 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
 Y W N D G H G G V T Y T N G P G
 TAT TGG AAC GAT GGC CAT GGT GGT GTT ACC TAT ACA AAC GGG CCC GGA
 ATA ACC TTG CTA CCG GTA CCA CCA CAA TGG ATA TGT TTG CCC GGG CCT
 NcoI XyTv-7 ApaI

 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
 G Q F S V N W S N S G N F V G G
 GGC CAA TTT AGC GTC AAT TGG TCT AAC TCC GGA AAC TTC GTA GGT GGA
 CCG GTT AAA TCG CAG TTA ACC AGA TTG AGG CCT TTG AAG CAT CCA CCT
 MunI BspEI

 TrX-3
 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64
 K G W Q P G T K N K V I N F S G
 AAA GGT TGG CAA CCC GGG ACC AAA AAT AAG GTG ATC AAC TTC TCT GGA
 TTT CCA ACC GTT GGG CCC TGG TTT TTA TTC CAC TAG TTG AAG AGA CCT
 XmaI TrX-6

 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
 S Y N P N G N S Y L S V Y G W S
 TCT TAT AAT CCG AAT GGG AAT TCA TAC TTA AGC GTC TAT GGC TGG TCT
 AGA ATA TTA GGC TTA CCC TTA AGT ATG AAT TCG CAG ATA CCG ACC AGA
 EcoRI AflII

 XyTv-4
 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95
 R N P L I E Y Y I V E N F G T
 AGA AAC CCA CTG ATT GAA TAT TAC ATT GTC GAA AAT TTC GGT AC
 TCT TTG GGT GAC TAA CTT ATA ATG TAA CAG CTT TTA AAG C
 Xba I XyTv-5 KpnI

FIGURE 2

XyTv-101

		92	93	94	95	96	97	98	99	100	101	102	103	104	105
V	D	N	F	G	T	Y	N	P	S	T	G	A	T	K	L
TC	GAC	AAT	TTC	GGT	ACC	TAC	AAT	CCG	AGT	ACC	GGC	GCC	ACA	AAA	TTA
3'-G	TTA	AAG	CCA	TGG	ATG	TTA	GGC	TCA	TGG	CCG	CGG	TGT	TTT	AAT	
SaII				KpnI			XyTv-110			KasI/NarI					

XyTv-102

106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121
G	E	V	T	S	D	G	S	V	Y	D	I	Y	R	T	Q
GGC	GAA	GTC	ACT	AGT	GAT	GGA	TCC	GTA	TAT	GAT	ATC	TAC	CGT	ACC	CAA
CCG	CTT	CAG	TGA	TCA	CTA	CCT	AGG	CAT	ATA	CTA	TAG	ATG	GCA	TGG	GTT
			SpeI			BamHI								XyTv-109	

TrX-103

122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137
R	V	N	Q	P	S	I	I	G	T	A	T	F	Y	Q	Y
CGC	GTT	AAT	CAG	CCA	TCG	ATC	ATT	GGA	ACC	GCC	ACC	TTT	TAT	CAG	TAC
GCG	CAA	TTA	GTC	GGT	AGC	TAG	TAA	CCT	TGG	CGG	TGG	AAA	ATA	GTC	ATG
MluI					Clai										

138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153
W	S	V	R	R	N	H	R	S	S	G	S	V	N	T	A
TGG	AGT	GTT	AGA	CGT	AAT	CAT	CGG	AGC	TCC	GGT	TCG	GTT	AAT	ACT	GCG
ACC	TCA	CAA	TCT	GCA	TTA	GTA	GCC	TCG	AGG	CCA	AGC	CAA	TTA	TGA	CGC
	TrX-108							SacI							

XyTv-104

154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
N	H	F	N	A	W	A	Q	Q	G	L	T	L	G	T	M
AAT	CAC	TTT	AAT	GCA	TGG	GCA	CAG	CAA	GGG	TTA	ACC	CTA	GGT	ACA	ATG
TTA	GTG	AAA	TTA	CGT	ACC	CGT	GTC	GTT	CCC	AAT	TGG	GAT	CCA	TGT	TAC
			NsiI			XyTv-107					AvrII				

XyTv-105

170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185
D	Y	Q	I	V	A	V	E	G	Y	F	S	S	G	S	A
GAT	TAT	CAA	ATC	GTA	GCG	GTG	GAA	GGC	TAC	TTC	TCG	AGT	GGT	TCC	GCT
CTA	ATA	GTT	TAG	CAT	CGC	CAC	CTT	CCG	ATG	AAG	AGC	TCA	CCA	AGG	CGA
						XyTv-106					XhoI				

186	187	188	189	190
S	I	T	V	S
AGT	ATT	ACA	GTG	AGC
TCA	TAA	TGT	CAC	TCG
			ATT	TCT
				AG-5'
				BglII

FIGURE 2 CONT'D

09990874-112101

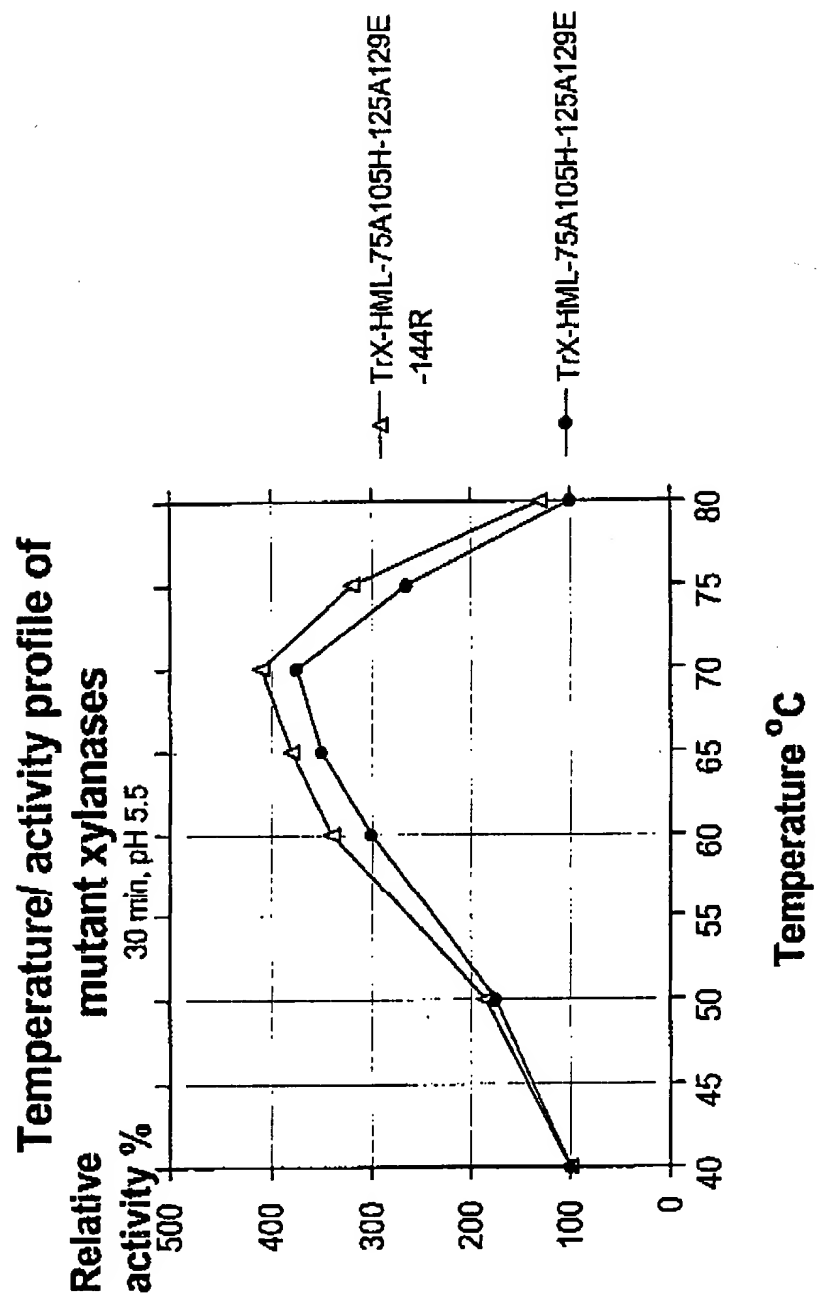


Figure 3

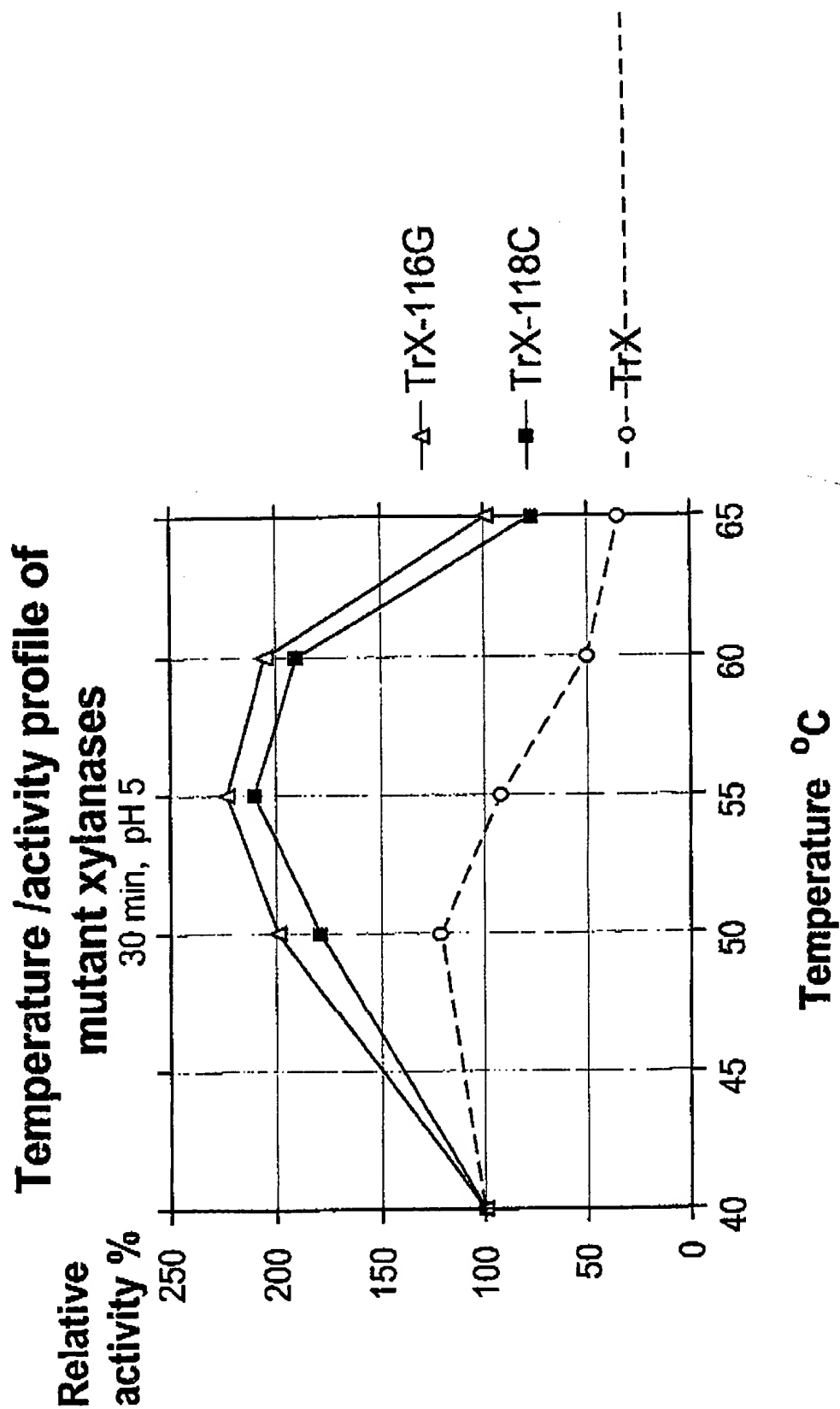


Figure 4

Effect of Temperature on the activity of mutant xylanase

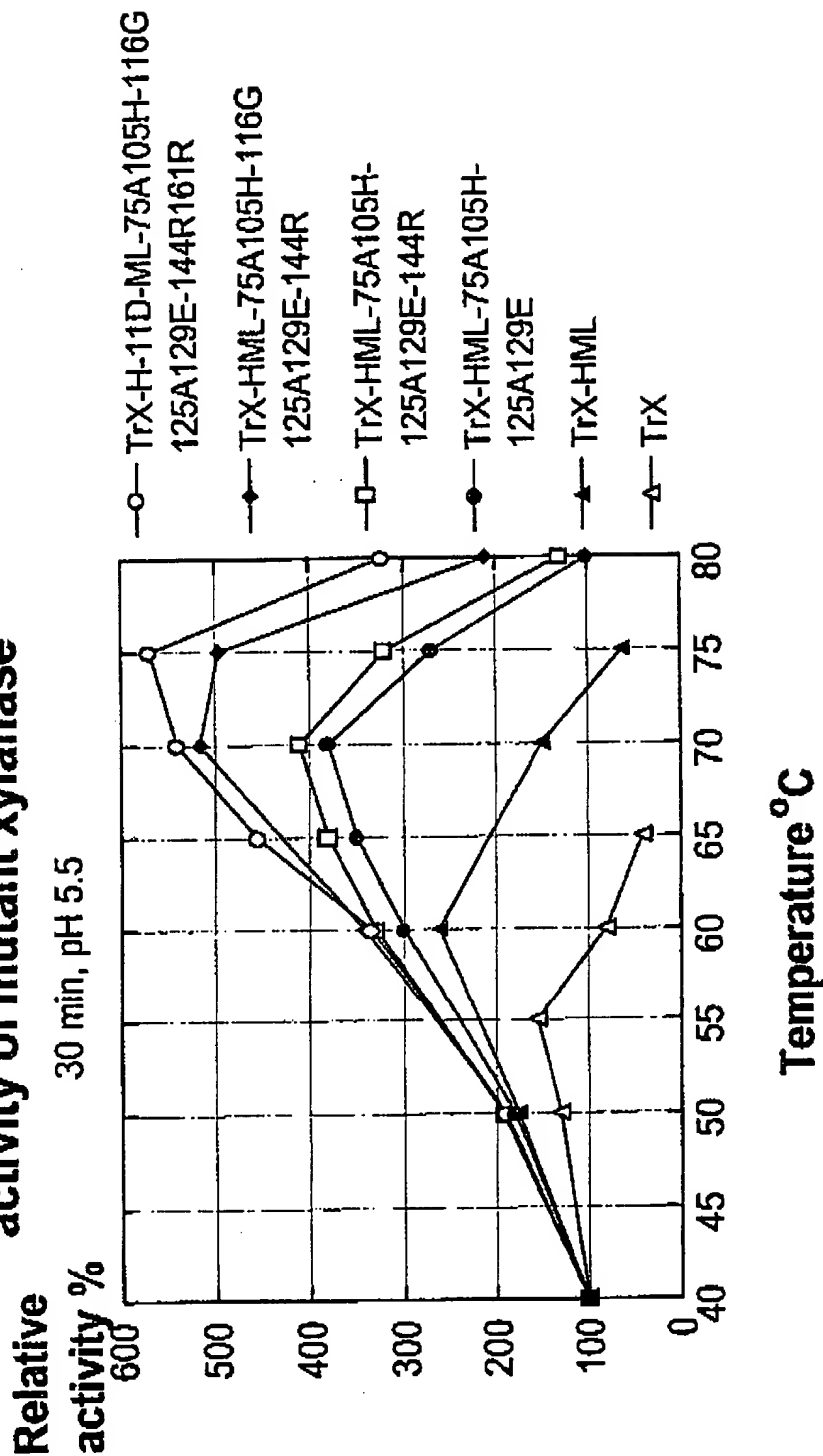


Figure 5

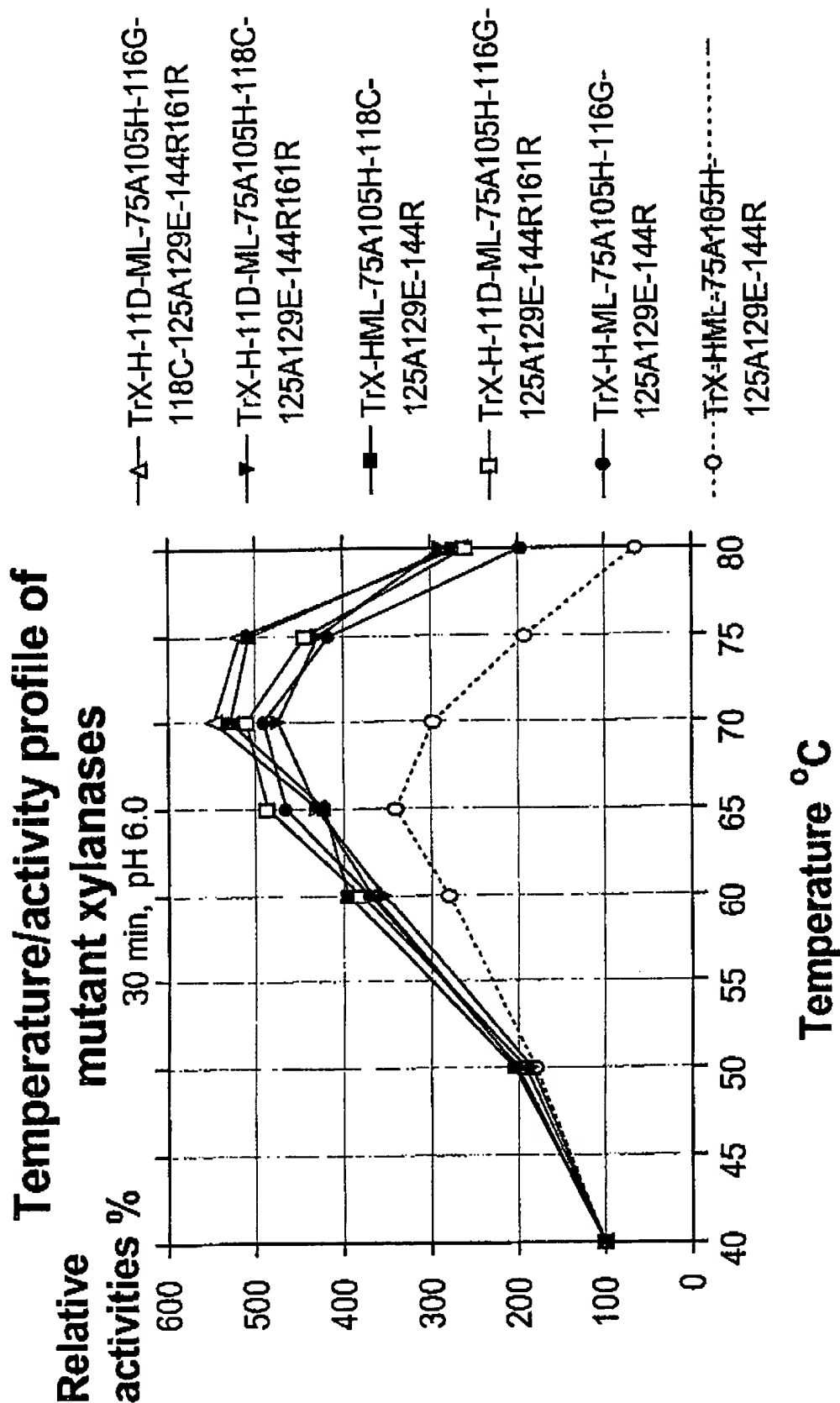


Figure 6

Temperature/activity profile of

Relative activities % mutant xylanases

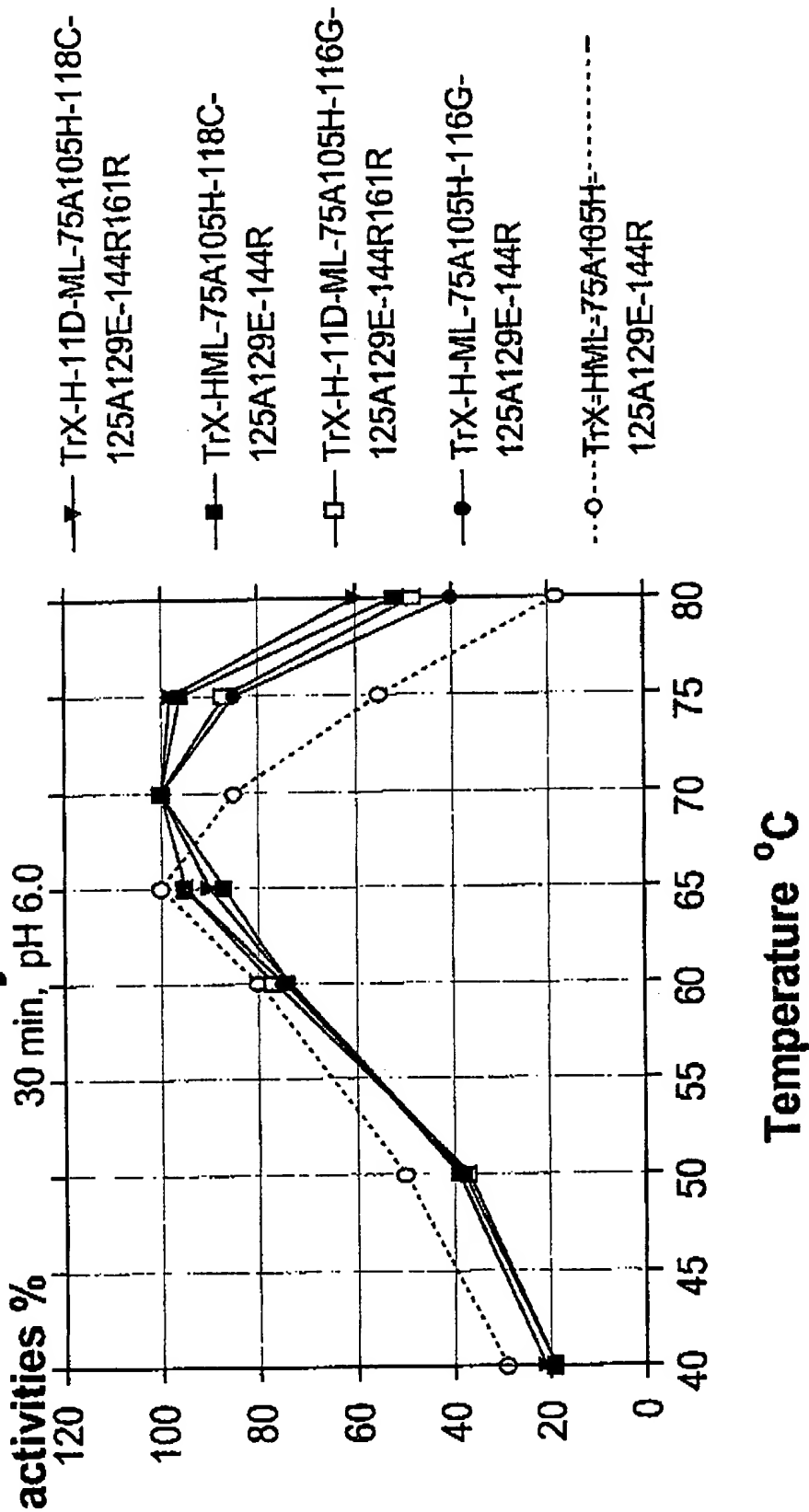


Figure 7

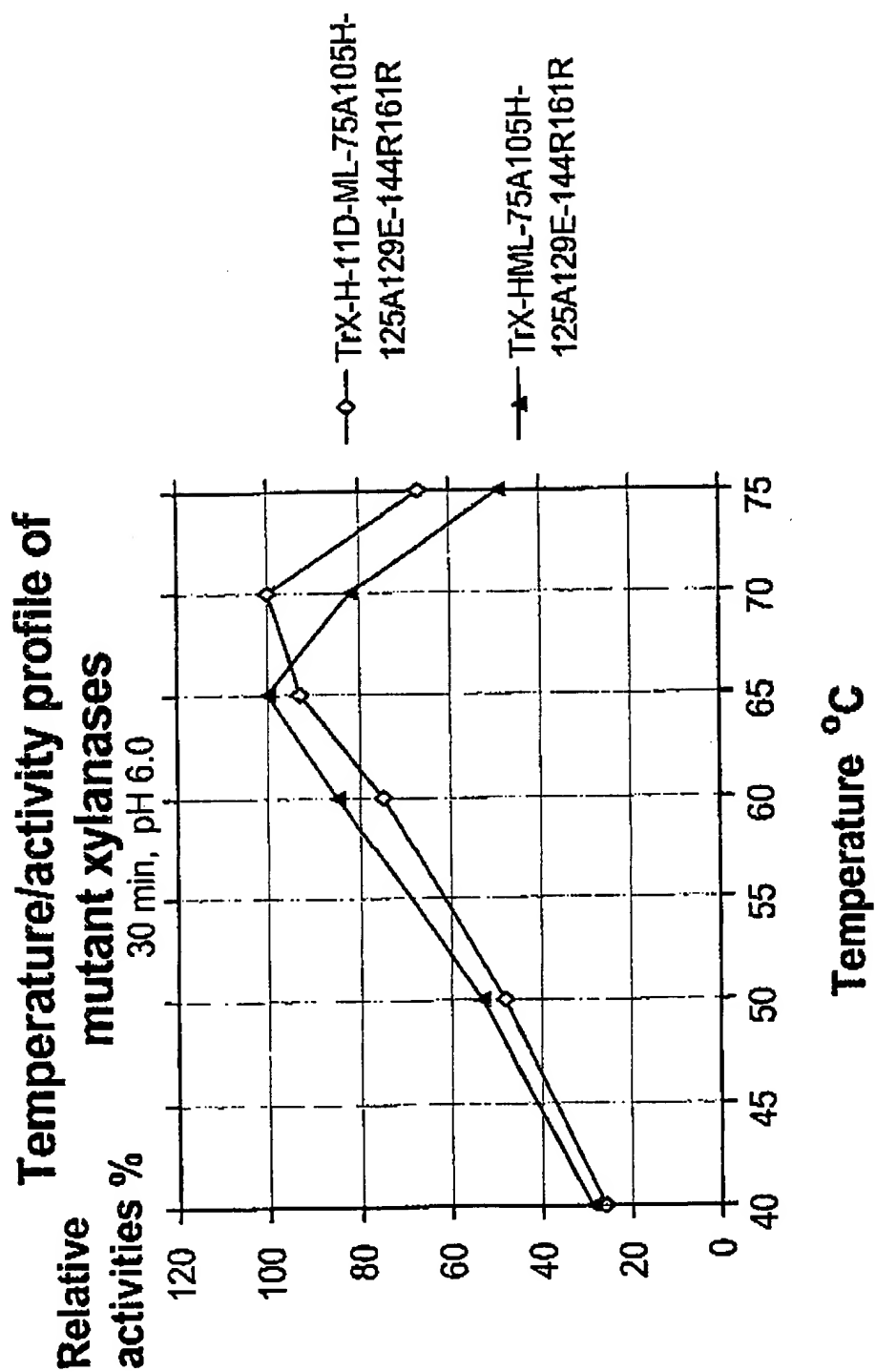


Figure 8

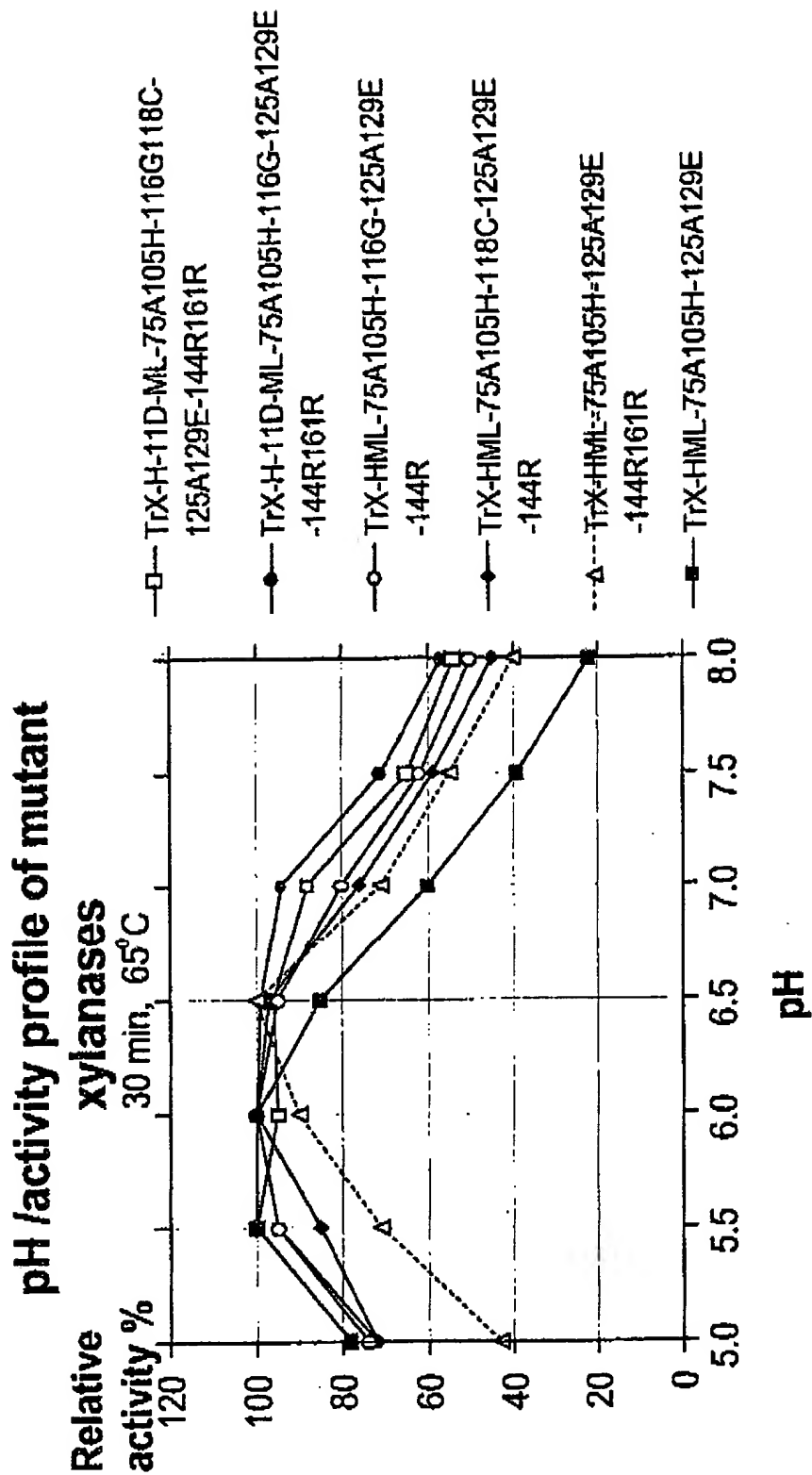


Figure 9

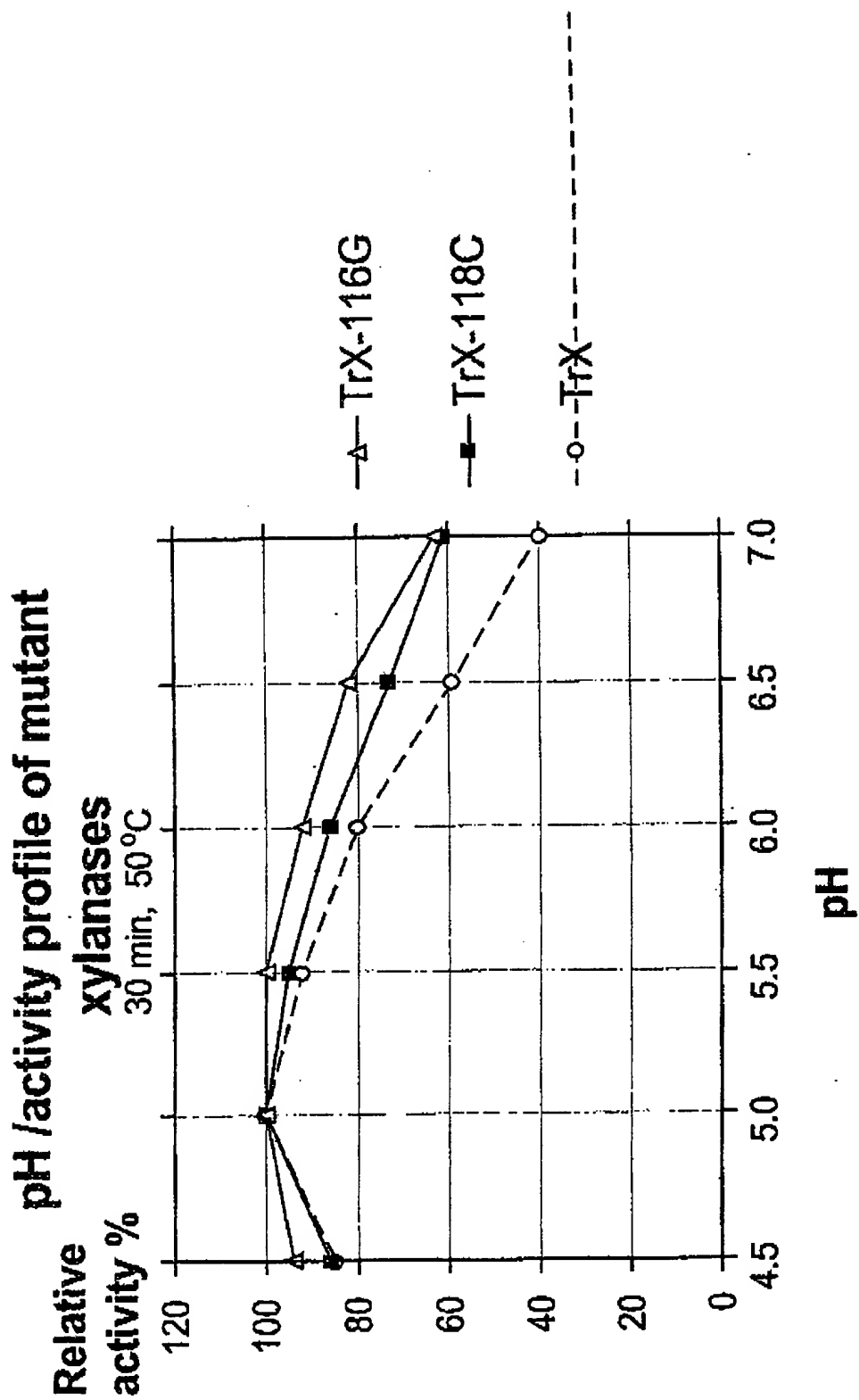


Figure 10

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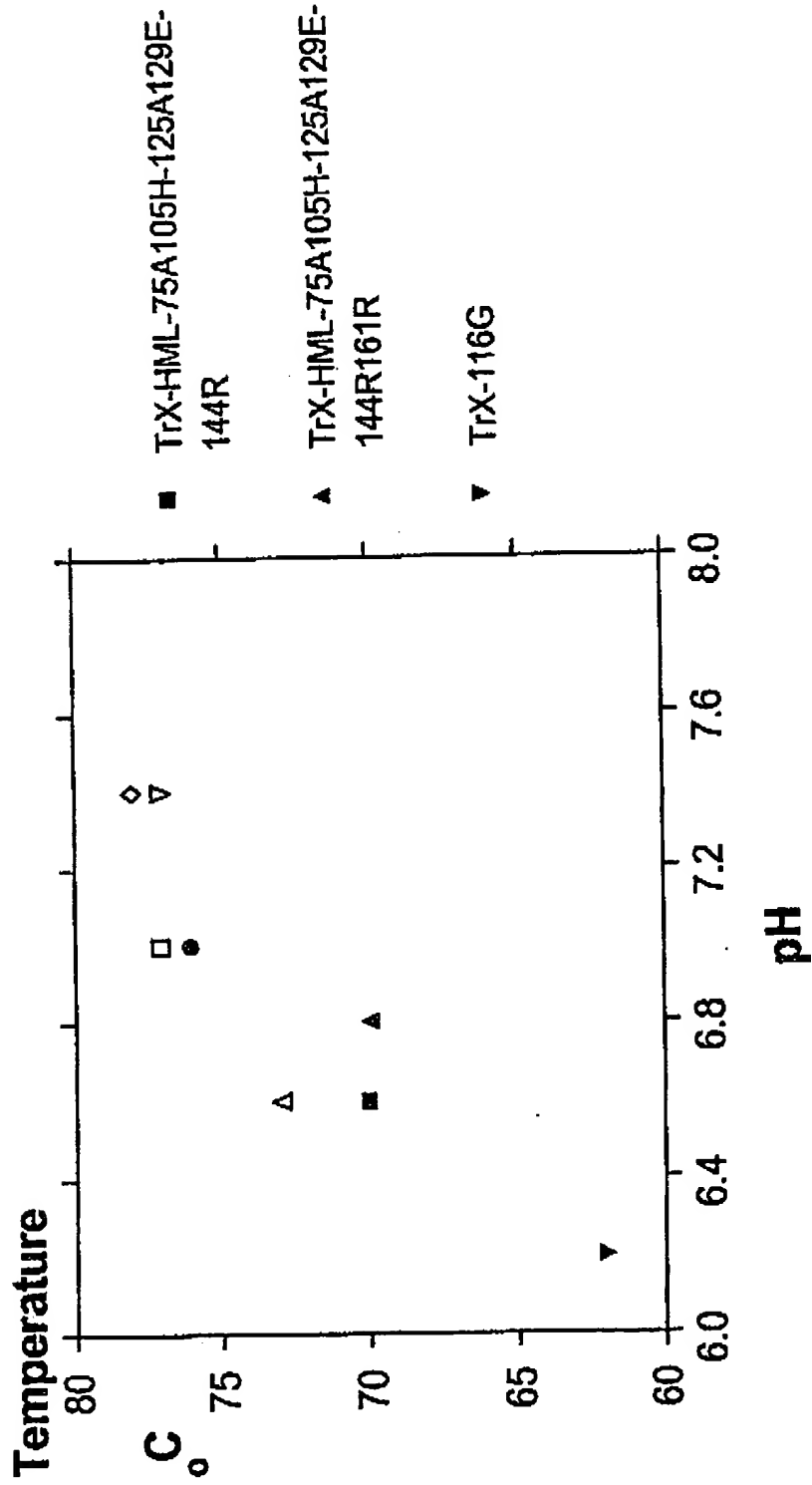


Figure 11